Genetic variation at the alcohol dehydrogenase locus in *Drosophila melanogaster:* A third ubiquitous allele

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Summary. A 3rd allele at the *Adh* locus, $Adh^{FCh.D.}$, has been found at polymorphic frequencies in natural populations of *D. melanogaster.* The ADH-FChD enzyme has properties distinct from those of the 2 more common forms of ADH. The *Adh* polymorphism should now be analyzed as a triallelic system.

Natural populations of *Drosophila melanogaster* are commonly polymorphic for 2 variants at the *Adh* locus, Adh^F and Adh^S, identified by a difference in the electrophoretic mobility of the enzymes they encode[2,3]. Three other very rare electrophoretic variants have also been described[4,5]. The 2 common alleles, Adh^S and Adh^F, are clinally distributed with Adh^S decreasing in frequency with increasing distance from the equator both in the Northern[6] and Southern Hemispheres[7,8]. Experiments to investigate this polymorphism have assumed that there are only 2 readily identifiable alleles at the *Adh* locus[9] at frequencies high enough to be taken into account.

We have described[10–14] a 3rd allele which encodes an alcohol dehydrogenase (ADH) of higher thermostability than the products of either common variant. We now report that this allele, $Adh^{FCh.D.}$ ('fast' Chateau Douglas), occurs at polymorphic frequencies in over half the Australian populations screened and hence we argue that the *Adh* polymorphism must be considered a 3 allele system.

ADH-FCh.D. has a substrate specificity like ADH-S, an electrophoretic mobility on cellulose acetate sheets like ADH-F, but much greater thermostability than either of the others[11,14].

The thermostability characteristic of ADH-FCh.D. has been used to develop a post-electrophoresis heat-treatment screening technique to identify ADH variants[7] with similar properties. We have found (table 1) that all 18 'heat-resistant' electrophoretically 'fast' variants isolated from 6 natural populations in Australia are similar to ADH-FCh.D. in heat stability and activity ratio (the ratio of ADH activity with 2-propanol to the activity with ethanol as substrate). 15 'heat-sensitive' electrophoretically 'fast' variants from the same populations, had similar properties to the enzyme from our standard Adh^F stock (table 1). An analysis of variance showed that the 'heat sensitive' and 'heat resistant' classes were each homogeneous with respect to activity ratio although there was some heterogeneity within the classes in activity, both before and after heat treatment, which we attribute to differences in genetic background. The 'heat-resistant' class has lower ADH activity than the 'heat-sensitive' class. Overall, these data, together with the earlier finding that the 2 other naturally occurring 'heat-resistant' 'fast' variants (isolated from populations in North America[15] and Europe[16]) have an activity ratio like ADH-FCh.D.[11], suggest that probably all such variants represent the $Adh^{FCh.D.}$ allele.

We have screened 62 Australian populations for $Adh^{FCh.D.}$ and found that 2 populations were monomorphic Adh^S, 24 were polymorphic for only Adh^F and Adh^S but a further 36 also contained $Adh^{FCh.D.}$ at frequencies averaging 4.9%. However, in some samples, frequencies of $Adh^{FCh.D.}$ as high as 16% have been found. There is no evidence for a clinal distribution in the frequency of $Adh^{FCh.D.}$ even though the enzyme encoded is similar in many properties (other than thermostability) to ADH-S[12,14]. $Adh^{FCh.D.}$ seems to be at highest frequencies at the mid-point of the cline in Adh^F, at least in the Southern Hemisphere[12].

These results will be described in detail elsewhere but the data obtained in the same season for 2 of the populations (table 2) emphasise the need to score for $Adh^{FCh.D.}$. The frequency of *Adh* 'fast' alleles does not differ significantly between the Yenda and Ebor populations ($\chi^2_1 = 0.52$, $p > 0.3$). However the $Adh^{FCh.D.}$ allele is not present in the Yenda population, whereas it has been found at a frequency of 0.16 in the Ebor population. Thus when the frequencies of $Adh^{FCh.D.}$ alleles are taken into account there is significant heterogeneity between the 2 populations in Adh^F frequency ($\chi^2_1 = 5.09$, $p < 0.05$).

This result points to the importance of screening natural populations for $Adh^{FCh.D.}$ to obtain true estimates of gene frequencies at the *Adh* locus, particularly in view of the marked difference in thermostability between ADH-FCh.D. and either ADH-F or ADH-S. These data are also relevant to the interpretation of laboratory studies on differential fitnesses of *Adh* genotypes. Only a few of the previous laboratory experiments on the *Adh* polymorphism

Table 1. Comparison of ADH derived from 'heat sensitive' and 'heat resistant' fast alleles (SE in parenthesis)

	No. of strains	ADH activity with propanol	Activity ratio*	ADH activity remaining in heat-treated extracts** (%)	
				40 °C	44 °C
Heat-sensitive 'fast' (ADH-F)	15	328.3 (4.8)	6.59 (0.09)	46.2 (1.57)	4.2 (0.59)
Heat-resistant 'fast' (ADH-FCh.D.)	18	131.3 (1.8)	4.16 (0.04)	94.6 (1.07)	45.7 (1.68)

*The activity ratio is the ratio of activity with 2-propanol to the activity with ethanol as substrate. ADH activity was measured in 1 ml reaction mixture which included 150 mM ethanol or 2-propanol and 2 mM NAD^+ in 100 mM sodium phosphate buffer, pH 7.5.

**Thermostability was measured by exposing 200 µl samples of crude extract in small test tubes to heat treatment in a water bath for 5 min at either 40 or 44 °C. Thermal denaturation was terminated by plunging the tubes into ice-cold water; ADH activity was then asayed with 2-propanol as substrate.

Table 2. Genotype frequencies at the *Adh* locus in samples from 2 populations in New South Wales, Australia

Population	Latitude (°S)	Longitude (°E)	Genotypes at *Adh* locus						Gene frequencies	
			$\frac{F}{F}$	$\frac{S}{S}$	$\frac{FCh.D.}{FCh.D.}$	$\frac{F}{S}$	$\frac{F}{FCh.D.}$	$\frac{S}{FCh.D.}$	Adh^F	$Adh^{FCh.D.}$
Yenda	34.1	146.1	42	17	–	40	–	–	0.62	–
Ebor	30.2	152.2	21	17	5	35	8	13	0.43	0.16

have used single allele lines[12,17]. Therefore much of the earlier data, particularly from population cages maintained at different temperatures and from comparative studies of the heat-resistancet properties of *Adh* variants, must now be viewed with caution.

There may well be further cryptic variation at the *Adh* locus which new techniques will reveal, although methods of sequential gel electrophoresis have not detected 'hidden' variation at this locus[18]. Nevertheless, it is clear that interpretation of the maintenance of the *Adh* polymorphism must now be in terms of a tri-allelic system[19].

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Inter-locus allozyme mobility correlations and species divergence

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Summary. An analysis of allozyme data from numerous sets of related vertebrate and *Drosophila* species shows that species divergence does not generally seem to be accompanied by an overall increase or decrease in enzyme charge. The 2 significant results came from vertebrate inter-generic comparisons.

The cellular environment of certain highly specialized tissues appears in several cases to favor the functioning of isozymes whose electrical charge and hence electrophoretic mobility is recognizably and generally different from the corresponding isoenzymes in other tissues. It has, for example, been pointed out that the creatine kinase and lactate dehydrogenase isozymes restricted to the neural tissue of advanced teleosts characteristically possess a high net negative charge[1], and this is also true of the fructose 16 biphosphate aldolase isozyme found in the eye and brain of many vertebrates (including fishes)[2]. It has further been suggested that the low net charge of serum albumin of the marine iguana *Amblyrhynchus cristatus* when compared with that of 2 species of land iguana may have been critical for successful reptilian adaptation to an aquatic environment[3], and that generally charge (and concentration) of albumin are important factors in controlling water loss in reptiles[4]. Thus in at least some instances adaptation of proteins to particular cellular or ecological environments may be accompanied by a change in charge.

If adaptation of a population to a new environment does favor a change in protein charge, then it is conceivable that there could be selection for a general charge increase (or decrease) in physiologically important enzymes. Thus in comparing related species which have diverged from a common ancestor and subsequently adapted to new environments, the average anodal mobility of proteins may differ significantly between species. That is, there may be mobility correlations over protein loci between species. This is the hypothesis tested here. It should be noted that the theory of neutral variation[5–7] would not predict such correlations.

Many studies have been published describing patterns of allozymic variation at appreciable numbers of loci in related species, and the majority of these studies employ an internally consistent notation indicating the relative mobilities of the screened allozymes. We have tested for correlations in allozyme mobility between related vertebrate species and related *Drosophila* species, using studies scoring a minimum of 15 loci and 15 individuals per locus. Most of the sources of data are given in an earlier study[8]. The relative mobility of the product of the most frequent allele in each species was used in the comparisons.

14 of the vertebrate surveys and 1 *Drosophila* survey compared just 2 related species, and here the numbers of loci at which the most frequent alleles differed in mobility ranged from 1 to 12. Such data give an inadequate data

Interspecific comparisons of allozyme mobility involving surveys screening 3 or more species

Group	N	χ^2	d.f.	p
Vertebrates, overall	30 (141)	120.74	111	0.2483
Icteridae[11]	1 (7)	14.42	6	0.0253
Cichlidae[12]	1 (6)	18.29	5	0.0026
Drosophila, overall	5 (27)	27.74	22	0.1845

N is the number of surveys analyzed, the number in parenthesis being the total number of species screened.